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Unrecognized Causes of Platelet Transfusion Failure in the Presence of Anti-HL-A Antibodies

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When in a patient who is receiving random donor platelet infusions there is an inadequate rise in platelet count and anti-human HL-A antibodies are noted in the serum, it is natural to assume that platelet destruction results from an immunologic cause. Nonimmunologic causes of such failure do occur, however. Two of the most common are disseminated intravascular coagulation and splenomegaly.

PLATELET TRANSFUSION has by now become well established as a means of treatment of thrombocytopenias of depressed platelet production, whether due to leukemia, aplastic anemia or chemotherapy of malignant disease. Whether harvested from the blood of random donors or obtained by plateletpheresis of selected donors, about 65 to 70 percent of the infused platelets should circulate, many of the others being isolated in the spleen. Maximum survival of transfused platelets should normally be eight to ten days.[1]

It has also been established that platelets contain human leukocyte antigen (HL-A) and other hereditary antigens. In a large percentage of patients receiving many transfusions of random donor platelets, sooner or later anti-HL-A antibodies will develop which destroy transfused platelets unless they are of compatible HL-A type. So when the response in a patient who is receiving random donor platelet infusions consists of an inadequate rise in platelet count, and anti-HL-A antibodies are shown in the serum, it is natural to assume that platelet destruction is occurring because of an immunologic cause.

There are nonimmunologic causes of failure of platelet transfusion, however; two of the more common of these are disseminated intravascular coagulation (DIC) and splenomegaly with splenic sequestration. When either of these events occurs in a patient in whom the presence of anti-HL-A antibodies can be shown, it is very easy to overlook the complicating event, and assume that platelet destruction is due to the HL-A antibodies.

In a recent and continuing study by the National Institutes of Health of patients who had received many platelet transfusions, and who had become resistant to random donor platelets, the author encountered several with such unexpected complications. The following two cases serve as characteristic examples.

Reports of Cases

CASE 1. A 35-year-old Mexican-American woman entered the hospital on February 27, 1975, with a diagnosis of chronic granulocytic leukemia in "blast crisis." Chronic granulocytic leukemia had been diagnosed three years before because of a massively enlarged spleen and characteristic

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ABBREVIATIONS USED IN TEXT

ACT = activated coagulation time
APTT = activated partial thromboplastin time
CGL = chronic granulocytic leukemia
DIC = disseminated intravascular coagulation
Factor II = prothrombin
Factor V = proaccelerin
Factor VIII = anti-hemophilic globulin
FDP = fibrin degradation products
HL-A = human leukocyte antigen
PT = prothrombin time
TT = thrombin time

blood and bone marrow features. A Philadelphia chromosome (Ph') and partial deletion of chromosome G were also noted. Treatment had been carried out with hydroxyurea, busulfan and allopurinol, and in about 3½ months a complete remission had been achieved, which had been maintained fairly well until very recently without maintenance therapy. A rising leukocyte count with increasing numbers of myeloblasts prompted readmission of the patient to hospital.

On physical examination at admission, pallor of the skin was noted. The spleen was palpable 5 cm and the liver 4 cm below the costal margins. The patient reported that her right arm was sore and weak. An infiltrative lesion on the tongue, felt to be leukemic, was noted. The leukocyte count was 79,000 per cu mm, with a large percentage of myeloblasts and promyelocytes; the hematocrit was 23.5 percent; platelets numbered 74,000 per cu mm. The bone marrow was intensely hypercellular with myeloblasts and promyelocytes predominating. There were considerable numbers of megakaryocytes, however.

Treatment with radiation to spleen and tongue, and intensive therapy with hydroxyurea was carried out. There was no response; the spleen increased in size and the myeloblast count rose. An attempt was made to determine the patient's HL-A type, without success, so when the falling platelet count reached a hazardous level, two platelet infusions were given, each of four units from two available half-siblings. There was essentially no rise in her platelet count. She was then given 10 units of platelets from random donors, again without significant rise in the count. At this time, the presence of HL-A antibodies was shown by positive lymphocytotoxicity tests against eight of a panel of ten donor lymphocytes.

Eventually HL-A typing was successfully done and the patient was found to be type HL-A 1, 9; W-5, W-22, and group O. No donors of this type could be found in the local panel of close to 2,500 HL-A typed donors, so we chose a group A donor of type HL-A 1, 9; 7, W-10, the first two antigens being in common with those of the patient, the latter two being "cross-reactors" with the patient's antigen W-22. Four units of this donor's platelets raised the platelet count 4,400 per cu mm per unit, when corrected for the patient's surface area—a fairly satisfactory response, but by the next morning only 400 per cu mm survived in the circulation.

At this point it was decided that, although it was risky, the only hope of prolonging this patient's life significantly was by splenectomy, possibly to be followed by more hydroxyurea. Since we were unable to obtain even reasonably matched platelets with which to prepare the patient for surgery, the procedure was undertaken without previous platelet transfusion. However, as soon as the splenic artery was ligated, 15 units of group O random donor platelets were rapidly infused. The blood loss, which had been troublesome during the first stages of the operative procedure, almost immediately ceased and gave no more trouble subsequently. The striking rise in the platelet count, from 17,000 preoperatively to 185,000 postoperatively, is shown in Figure 1. Although fluctuating considerably subsequently, the count never again fell into bleeding levels. The patient died one week postoperatively in spite of postoperative hydroxyurea. Results of postmortem examination confirmed widespread infiltration with myeloblasts, but no recent abnormal bleeding.

CASE 2. A 55-year-old man was admitted to hospital with acute myeloblastic leukemia. On physical examination no massive splenomegaly or hepatomegaly was noted. Findings on laboratory studies showed progressive normoblastic anemia and grossly elevated leukocyte count, most of the cells being myeloblasts.

Under intensive chemotherapy with cytosine arabinoside and thioguanine, the leukocyte count fell to 1,500 per cu mm, with no myeloblasts, and the platelet count fell to 5,000 per cu mm, the bone marrow becoming extremely hypocellular. Transfusions of packed red cells supported the falling hemoglobin, and many units of random donor platelets supported the platelet count. Remission eventually occurred, and the patient left the hospital.

He returned for consolidation therapy, and there was no response to transfusions of random donor platelets. Antibody tests showed that a broad spectrum of anti-HL-A antibodies had developed. HL-A typing showed the patient to be to be HL-A 2, 10; W-5, W-16. We fortunately had in our file a donor of the same blood group and HL-A type, and four units of this donor's platelets caused a very significant rise in the patient's platelet count (Figure 2). He again left the hospital with moderate neutropenia and thrombopenia, but in early remission.

He returned seven months later, in a septic condition and with an extremely low platelet count. Hemostatic studies showed a fibrinogen of 250 mg per 100 ml (normal: 200 to 400), and an activated partial thromboplastin time (APTT) of 19.9 seconds (control: 33.9 seconds). Appropriate antibiotics were given, and 4 more units of platelets from the same HL-A identical donor. The platelet infusion this time caused a very small increment, but the patient responded to treatment of sepsis, and was again discharged.

He returned three months later, again in a septic condition and in relapse of the acute leukemia. Persistent epistaxis and a platelet count of only 7,000 per cu mm were noted. This time, the response to infusion of 4 units of platelets from the same HL-A matched donor was negative, the platelet count 20 hours after infusion having fallen from 7,000 to 2,000 per cu mm. Suspecting DIC due to sepsis, we initiated coagulation studies, but prothrombin time (PT) and thrombin time (TT) proved to be normal, and quantitative fibrinogen 275 mg per 100 ml. Fibrin degradation products ranged from 10 to 40 μg per ml—not considered a diagnostic level. The only abnormal finding on a hemostatic test, other than the platelet count, was the activated partial thromboplastin time of 22.5 seconds (control: 33.2 seconds).

In spite of the far from classical features of DIC, the clinician in charge agreed to light administration of heparin, feeling that in this preterminal patient, the survival of matched platelets might be significantly improved if the apparent DIC could be controlled. The patient therefore received 2,500 units of heparin, or about 35 units per kg of body weight, each four hours (15,000 units, or 210 units per kg of body weight per 24 hours). Three days after the previous platelet infusion, and two days after the start of heparin therapy, he again received four units of platelets from the same HL-A identical donor. The sepsis had remained essentially unchanged, but following the platelet infusion the nasal blood loss stopped and the platelet count rose from 3,000 to 14,000 per cu mm, and in the subsequent two days of heparin administration, to 28,000 per cu mm. The sepsis proved unmanageable, however, and the patient died a few days later, without active blood loss.

Discussion

In Case 1, although the HL-A antibodies undoubtedly were of importance, the rate of platelet destruction was tremendously hastened by the huge spleen, as shown by the increased survival after its removal. It is possible that the spleen had been producing a significant portion of the HL-A antibodies, as well as being the site of sequestration and destruction of most of the antibody-

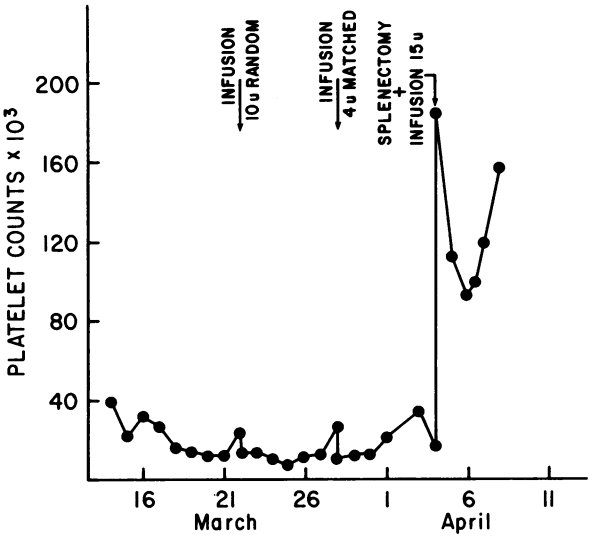

Figure 1.—Splenomegaly and survival of transfused random donor platelets in presence of anti-HL-A antibodies.

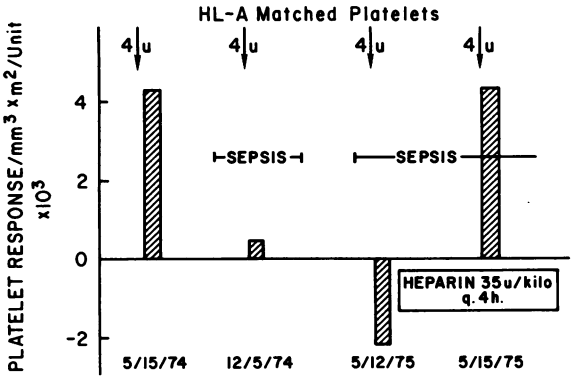

Figure 2.—Disseminated intravascular coagulation in terminal leukemia: Treatment with heparin and HL-A matched platelets.

injured platelets, because at examination it was found that considerable numbers of lymphocytes persisted in the spleen.

Although splenectomy of patients with massive spleens has long been known as a successful means of correcting thrombocytopenia due to hypersplenism,[2] we have found no reference in the literature to splenectomy in persons with a broad spectrum of anti-HL-A antibodies. Perhaps when faced with a patient with depressed thrombopoiesis, anti-HL-A antibodies and an absence of matched donors, one should not give up too readily. If the spleen is enlarged, its removal may allow additional time in which to explore further therapeutic methods.

The second patient presented a very different problem. The characteristic laboratory features of DIC, as widely recognized in recent years, include a decrease of those coagulation factors that are consumed in the production of a clot. The patient's blood may prove entirely incoagulable, due to a complete absence of fibrinogen, and assay findings show sharp decreases in factors VIII (antihemophilic globulin), II (prothrombin) and V (proaccelerin), as well as platelets. If the blood is not incoagulable, one characteristically encounters pronounced prolongation of the activated coagulation time (ACT), as well as the APTT, the PT and the TT. In addition, one finds strongly positive tests for fibrin monomers and fibrin degradation products (FDP). If fibrinogen survival studies are available, fibrinogen survival is found to be shortened. Thomas,[3] in a study of 58 patients with leukemia, found that 36 of them (62 percent) had episodes of blood loss associated with lowered fibrinogen and factor V, as well as platelets.

Gralnick and co-workers,[4,5] however, as well as Salvatori and co-workers[6] have reported that the laboratory features of DIC may differ sharply in patients with acute promyelocytic leukemia. They found APTT often abnormally *shortened,* the levels of fibrinogen and factor VIII varying from low to high, in spite of shortened fibrinogen survival. The PT and TT were usually normal, and fibrin monomer and FDP tests were not strongly positive. At times, the diagnosis of DIC proved very difficult to establish, but such patients, who had responded very poorly to platelet transfusions, showed normal increments when therapy with heparin was carried out before they received the platelets. Gralnick and Tan[5] suggested that such changes, although most characteristic of promyelocytic leukemia, occurred "more modestly" in patients with other forms of acute leukemia.

The patient in case 2 well illustrates the thesis of Gralnick and associates[4,5] that when platelet production is depressed, the laboratory picture of DIC may be very atypical. The patient's fibrinogen level was within usually accepted normal limits, although low for his physiologic state, since acute leukemias, particularly those with sepsis, tend to have abnormally high fibrinogen levels. Further, the APTT proved notably shortened on two occasions, as reported by Gralnick and Tan[5] in many of their patients. It is evident that when one encounters an undiagnosed thrombocytopenia, an APTT as well as fibrinogen level determination should be added to the panel of tests. If the fibrinogen level is decreased and the APTT prolonged, there is clear evidence of DIC. Likewise, if the APTT is abnormally shortened, as in this case, the evidence for DIC is strong, regardless of the fibrinogen level.

The most striking proof that failure of platelet infusions in this patient, in spite of the anti-HL-A antibodies, was not due entirely to isoimmunization was the fact that when he received HL-A identical platelets, they were destroyed rapidly. However, another similar infusion, given after heparin therapy had been carried out, caused a very satisfactory *rise* in the platelet count. The only possible explanation of this sharp change on low-dose administration of heparin is the assumption that the patient indeed had DIC.

One must remember, of course, that heparin therapy in a patient with severe thrombocytopenia involves appreciable hazard, and must not be undertaken lightly. On the other hand, platelet infusion in the face of DIC without heparin control is usually worthless. Judging by the results of the previous infusion in this patient, further infusion of matched platelets without control of the DIC would have proven valueless.

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